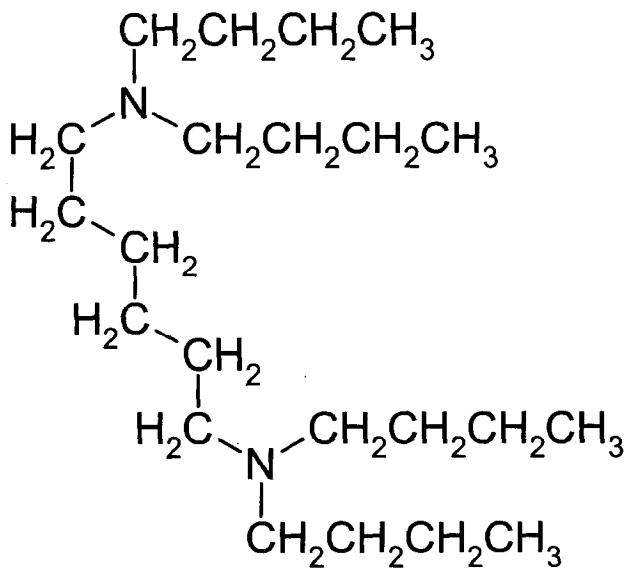
HPV Data Set

Tetrabutylhexamethylenediamine
CAS Number 27090-63-7



Existing Chemical	:	ID: 27090-63-7
CAS No.	:	27090-63-7
EINECS Name	:	N,N,N',N'-tetrabutylhexane-1,6-diamine
EC No.	:	248-219-2
Common name	:	TBHMD
Molecular Formula	:	$C_{22}H_{48}N_2$

Producer related part

Company	:	Solutia Inc, St. Louis MO
Creation date	:	06.01.2004

Substance related part

Company	:	Toxicology and Regulatory Affairs Freeburg IL, 62243 rauckman@toxicsolutions.com
Creation date	:	06.01.2004
Printing date	:	13.03.2005
Revision date	:	
Date of last update	:	10.03.2005
Number of pages	:	21

1.0.1 APPLICANT AND COMPANY INFORMATION

1.2 SYNONYMS AND TRADENAMES

1,6-Hexanediamine, N,N,N',N'-tetrabutyl- (8CI 9CI)

07.01.2004

N,N,N',N'-Tetrabutylhexamethylenediamine

07.01.2004

TBHMD

07.01.2004

Tetrabutylhexamethylenediamine

07.01.2004

2.1 MELTING POINT

Value	:	< -18 °C
Sublimation	:	
Method	:	
Year	:	
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Conducted by a standard method at the manufacturing plant.
Reliability	:	(2) valid with restrictions Data obtained by a scientifically defensible method.
Flag	:	Critical study for SIDS endpoint

07.01.2004 (8)

2.2 BOILING POINT

Value	:	= 83 °C at 2.9 hPa
Decomposition	:	
Method	:	
Year	:	
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Conducted by a standard method at the manufacturing plant.
Remark	:	The estimated boiling point at 1013 hPa from EPIWIN is: Boiling Pt (deg C): 380.18 (Adapted Stein & Brown method)
Reliability	:	(2) valid with restrictions Data obtained by a scientifically defensible method.
Flag	:	Critical study for SIDS endpoint

07.01.2004 (8)

2.3 DENSITY

Type	:	relative density
Value	:	= .82 at 24 °C
Method	:	
Year	:	
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Reliability	:	(2) valid with restrictions Data obtained by a scientifically defensible method.

07.01.2004 (8)

2.4 VAPOR PRESSURE

Value	:	ca. .000033 - .015 hPa at 25 °C
Decomposition	:	
Method	:	
Year	:	
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4

Remark :

VP: Using the MPVPBP program and correcting the EPIWIN estimate by the factor it under predicts the measured VP at 83 deg C (344), the corrected predicted VP at 25 deg C is 0.015 hPa based on a boiling point of 83 C at 2.2 mm Hg. This value has been added as the top of the VP range in the IUCILD entry. It is assumed that the measured vapor pressure is correct and this value for VP (0.015 hPa) is used in the fugacity calculations

Reliability : (2) valid with restrictions

Estimates made by an accepted method are assigned a reliability score of 2.

Flag : Critical study for SIDS endpoint

07.01.2004 (4)

2.5 PARTITION COEFFICIENT

Partition coefficient	:	octanol-water
Log pow	:	ca. 4.6 - 7.6 at 25 °C
pH value	:	ca. 9 - 11
Method	:	other (calculated)
Year	:	
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4

Method :

Octanol water partition coefficients for the three major forms of TBHMD were obtained through the KOWWIN program (v1.66) by entering the structure of the component into the program using the SMILES code. This program estimates the partition coefficient by summing the coefficients of all fragments of the molecule based on an empirical equation that has been validated.

As TBHMD is expected to exist in the free-base (unionized), the mono-protonated form and the di-protonated form in solution at nominal pH levels, the Ko/w was calculated for all three forms.

Result :

KOWWIN Program (v1.66) Results:
==========

Log Kow(version 1.66 estimate): 7.59 free base form
Log Kow(version 1.66 estimate): 6.08 N+ form
Log Kow(version 1.66 estimate): 4.56 N+N+ form

SMILES : CCCCN(CCCC)CCCCCCN(CCCC)(CCCC)
CHEM : TBHMD
MOL FOR: C22 H48 N2
MOL WT : 340.64

TYPE	NUM	LOGKOW FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	4	-CH3 [aliphatic carbon]	0.5473	2.1892
Frag	18	-CH2- [aliphatic carbon]	0.4911	8.8398
Frag	2	-N< [aliphatic attach]	-1.8323	-3.6646
Const		Equation Constant		0.2290
			Log Kow =	7.5934

SMILES : CCCCN(CCCC)CCCCCCN(CCCC)(CCCC)(H)
CHEM : TBHMD-H+ (charged form)
MOL FOR: C22 H49 N2
MOL WT : 341.65

TYPE	NUM	LOGKOW FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	4	-CH3 [aliphatic carbon]	0.5473	2.1892
Frag	18	-CH2- [aliphatic carbon]	0.4911	8.8398
Frag	1	-N< [aliphatic attach]	-1.8323	-1.8323
Frag	1	>N< [+5 valence; single bonds; H attach]	-4.6000	-4.6000
Factor	1	Reaction: nitrogen[+5] / polar group	1.2500	1.2500
Const		Equation Constant		0.2290
			Log Kow =	6.0757

SMILES : CCCCN(H)(CCCC)CCCCCCN(CCCC)(CCCC)(H)
CHEM : H-TBHMD-H++ (twice charged form)
MOL FOR: C22 H50 N2
MOL WT : 342.65
MANUAL CALCULATION EPIWIN WOULD ONLY ADD IN ONE CHARGED NITROGEN

TYPE	NUM	LOGKOW FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	4	-CH3 [aliphatic carbon]	0.5473	2.1892
Frag	18	-CH2- [aliphatic carbon]	0.4911	8.8398
Frag	2	>N< [+5 valence; single bonds; H attach]	-4.6000	-9.2000
Factor	2	Reaction: nitrogen[+5] / polar group	1.2500	2.500
Const		Equation Constant		0.2290
			Log Kow =	4.5580

Conclusion :

Depending on the solution pH, the material will exist in solution in one of three forms ranging in Log Ko/w from 4.6 to 7.6. All three are expected to be present from about pH 9 to 11. Above or below this pH range, one form will predominate.

Reliability : (2) valid with restrictions

Estimates made by an accepted method are assigned a reliability score of 2.

Flag : Critical study for SIDS endpoint

07.01.2004 (7)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	= 1.2 g/l at °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	10.25 at 25 °C
Description	:	moderately soluble (100-1000 mg/L)
Stable	:	yes
Method	:	Conducted by a standard method at the manufacturing plant.
Remark	:	

Solubility of amines is a function of pH, this value is only accurate for solubility in pure water. This value can be confirmed by calculations based on pH and typical pKa values for tertiary amines and suggests that the material has a actual pKa of about 10.25.

The EPIWIN predicted solubility is of the material is only 0.05 g/L. On the other hand, the ionized form should be more soluble. If it is assumed that as material is dissolved, it is ionized until the pH of the solution increases to the pKa for the material, at which point the material in solution will be half ionized (the definition of the pKa). Then 0.12 g/L or 3.53 exp-4 M is in solution and 1.765 exp-4 M is ionized. This would put the hydrogen ion concentration at 5.67 exp-11 M, which corresponds to a pH for the solution of 10.25.

Although the pKa for TBHMD has not been measured, it is predicted to be 10.17 by the SPARC program, as this is very close to the calculated solution pH as the material approaches its reported solubility limit in pure water, it provides confirmation that the measured solubility of 0.12 grams per liter is a correct value for pure water.

The actual solubility under environmental conditions will be dependent on the starting pH of the water and its buffering capacity. Thus, under most environmental conditions, 0.12 g/L should be considered a lower limit on solubility.

Reliability	:	(2) valid with restrictions

Data obtained by a scientifically defensible method.

Flag	:	Critical study for SIDS endpoint

07.01.2004 (5) (8)

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	1500000 molecule/cm³
Rate constant	:	ca. .000000000213 cm³/(molecule*sec)
Degradation	:	ca. 50 % after .6 hour(s)
Deg. product	:	
Method	:	
Year	:	
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4

Method :

The structure was initially examined to determine if there was a chromophore that could absorb light energy at wavelengths above 295 um. As there is not, it was assumed that direct photolysis would be unimportant to the fate of the test material.

The APOWIN program was also run to determine an estimated rate of reaction with hydroxyl radical. This rate was used to estimate the half-life of TBHMD in the troposphere assuming a tropospheric hydroxyl radical concentration of 1,500,000 molecules hydroxy radical per cm3.

Result :

The calculated half-life is 0.6 hours based on 1,500,000 molecules of hydroxyl radical per cc.

```
AOP Program (v1.90) Results:
============================
SMILES : CCCCN(CCCC)CCCCCCN(CCCC)(CCCC)
CHEM   : TBHMD
MOL FOR: C22 H48 N2
MOL WT : 340.64

----- SUMMARY (AOP v1.90): HYDROXYL RADICALS---------
Hydrogen Abstraction       =  80.6730 E-12 cm3/molecule-sec
Reaction with N, S and -OH = 132.0000 E-12 cm3/molecule-sec
Addition to Triple Bonds   =   0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =   0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =   0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings    =   0.0000 E-12 cm3/molecule-sec

   OVERALL OH Rate Constant = 212.6729 E-12 cm3/molecule-sec
   HALF-LIFE =     0.050 Days (12-hr day; 1.5E6 OH/cm3)
   HALF-LIFE =     0.604 Hrs
```

Conclusion :

A value of approximately 0.6 hours is accepted as the atmospheric half-life of TBHMD in the troposphere due to indirect photolysis. No direct photolysis or reaction with atmospheric ozone is anticipated.

Reliability	:	(2) valid with restrictions Estimates made by an accepted method are assigned a reliability score of 2.
Flag	:	Critical study for SIDS endpoint

31.10.2004 (1)

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	at °C
t1/2 pH7	:	at °C
t1/2 pH9	:	at °C
Degradation	:	< 50 % after 1 year at pH and °C
Deg. product	:	
Method	:	
Year	:	
GLP	:	
Test substance	:	other TS

Method :

The stability of this material in water is estimated based on established chemical principles.

Result :

Although amines are potentially susceptible to hydrolysis*, experience suggests that these simple tertiary amines are resistant to hydrolysis. The only plausible mechanism for hydrolysis is protonation of the amine to the nitrogen-centered cation followed by Sn1 elimination of a carbocation with the charge residing on a primary carbon. As primary carbocations are poor leaving groups, this reaction is considered unlikely under normal environmental conditions.

The presence of a second tertiary amine center is not expected to influence the water stability of the compound as it is situated several carbons away.

Support for the hydrolytic stability of TBHMD also comes from thermodynamic considerations. The enthalpy of reaction for hydrolysis of a tertiary amine to a secondary amine and butyl alcohol is calculated by summing the strengths of bonds broken and subtracting the sum of the strengths of the bond formed. (Organic Chemistry by Peter Vollhardt, W.H. Freeman & Co, NY, NY 1987 pp71-73)

Bonds broken	
Water O-H	497 kJ
N-C	350 kJ

Bonds formed	
Alcohol C-OH	-356 kJ
Amine N-H	-382 kJ

Total estimated enthalpy of reaction = +109 kJ/mole

As the enthalpy of reaction indicates a significantly endothermic reaction and the transition state for hydrolytic reaction (primary butyl cation) is

relatively high energy (as compared to a tert-butyl cation, for example), this hydrolysis reaction is considered unlikely under environmental conditions.

Bond energies from Lide, Handbook of Chemistry 84th edition 2003-2004 section 9

* The aliphatic amine moiety is considered potentially susceptible to hydrolysis by Harris (J.C. Harris in Lyman W, Reehl, W and Rosenblat, D. Handbook of Chemical Property Estimation Methods. American Chemical Society, Washington D.C. 1990, page 7-6).

Test substance : Tetrabutylhexamethylenediamine (TBHMD) CASNO 27090-63-7

Conclusion :

Experiences with tertiary amines with similar structures indicate hydrolytic stability. Thermodynamic calculations of the enthalpy of hydrolysis also indicate that hydrolysis is an endothermic reaction. As the transition state is also considered to have a high delta G, hydrolysis of TBHMD is considered highly unlikely under environmental conditions. It can be concluded that TBHMD stable in water and has a hydrolysis half-life of greater than 1 year.

Reliability : (2) valid with restrictions

Estimates made by an accepted method are assigned a reliability score of 2.

Flag : Critical study for SIDS endpoint

09.03.2005 (2)

3.3.2 DISTRIBUTION

Media : other: air soil sediment and water

Method : Calculation according Mackay, Level III

Year :

Method :

Measured values for physical properties of TBHMD were input into EPIWIN as shown below. Default biodegradation rates were determined to be reasonable. Model was set to an initial distribution of 100% to water due to the material's low volatility and use pattern. The EQC Level 3 model (as found in EPIWIN 3.05) was utilized.

This material is an amino compound and the EC Level 3 model will not adequately handle the equilibrium states between the charged (protonated) forms and the uncharged form of the material. Because of this, the distribution was independently calculated for each form realizing that the actual distribution will be a pH dependent composite of these three calculations.

Result :

```
Level III Fugacity Model (Full-Output):
=======================================
  Chem Name   : TBHMD
  Molecular Wt: 340.64
  Henry's LC  : 5.6e-005 atm-m3/mole (calc VP/Wsol)
  Vapor Press : 0.015 mm Hg  (user-entered)
  Log Kow     : 7.59  (Kowwin program)
  Soil Koc    : 1.6e+007  (calc by model)
```

	Concentration (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.000311	1.21	0
Water	10.5	360	1000
Soil	1.01e-005	360	0
Sediment	89.5	1.44e+003	0

	Fugacity (atm)	Reaction (kg/hr)	Advection (kg/hr)	Reaction (percent)	Advection (percent)
Air	2.95e-015	2.36	0.0411	0.236	0.00411
Water	4.24e-012	266	138	26.6	13.8
Soil	3.19e-021	0.000258	0	2.58e-005	0
Sediment	1.27e-012	569	23.6	56.9	2.36

Persistence Time: 1.32e+003 hr
Reaction Time: 1.58e+003 hr
Advection Time: 8.15e+003 hr
Percent Reacted: 83.8
Percent Advected: 16.2

Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):
Air: 1.207
Water: 360
Soil: 360
Sediment: 1440
Biowin estimate: 3.130 (weeks)

Advection Times (hr):
Air: 100
Water: 1000
Sediment: 5e+004

Level III Fugacity Model (Full-Output):

Chem Name : TBHMD-H+ (charged form)
Molecular Wt: 341.65
Henry's LC : 1.41e-013 atm-m3/mole (Henrywin program)
Vapor Press : 4.54e-009 mm Hg (Mpbpwin program)
Log Kow : 6.08 (Kowwin program)
Soil Koc : 4.93e+005 (calc by model)

	Concentration (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	2.73e-013	1.75	0
Water	29.8	208	1000
Soil	6.42e-009	208	0
Sediment	70.2	832	0

	Fugacity (atm)	Reaction (kg/hr)	Advection (kg/hr)	Reaction (percent)	Advection (percent)
Air	5.19e-027	5.73e-010	1.45e-011	5.73e-011	1.45e-012
Water	1.81e-019	525	158	52.5	15.8
Soil	6.59e-032	1.13e-007	0	1.13e-008	0
Sediment	3.24e-020	310	7.44	31	0.744

```
Persistence Time: 529 hr
Reaction Time:    634 hr
Advection Time:   3.21e+003 hr
Percent Reacted:  83.5
Percent Advected: 16.5

Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):
   Air:      1.75
   Water:    208.1
   Soil:     208.1
   Sediment: 832.3
     Biowin estimate: 3.383  (days-weeks)

Advection Times (hr):
   Air:      100
   Water:    1000
   Sediment: 5e+004



=====================================
 Chem Name   : H-TBHMD-H++  (twice charged form)
 Molecular Wt: 342.66
 Henry's LC  : 8.91e-015 atm-m3/mole (Henrywin program)
 Vapor Press : 5.29e-013 mm Hg  (Mpbpwin program)
 Log Kow     : 4.56  (user-entered)
 Soil Koc    : 1.49e+004  (calc by model)

        Concentration   Half-Life    Emissions
          (percent)        (hr)       (kg/hr)
 Air       1.88e-011       3.18         0
 Water     88.5            208          1000
 Soil      9.72e-010       208          0
 Sediment  11.5            832          0

           Fugacity    Reaction    Advection   Reaction    Advection
            (atm)      (kg/hr)      (kg/hr)    (percent)   (percent)
 Air       2.01e-029    1.04e-008   4.79e-010   1.04e-009   4.79e-011
 Water     2.86e-020    750         225         75          22.5
 Soil      9.98e-033    8.23e-009   0           8.23e-010   0
 Sediment  5.29e-021    24.3        0.583       2.43        0.0583

 Persistence Time: 254 hr
 Reaction Time:    329 hr
 Advection Time:   1.13e+003 hr
 Percent Reacted:  77.4
 Percent Advected: 22.6

 Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):
    Air:      3.182
    Water:    208.1
    Soil:     208.1
    Sediment: 832.3
      Biowin estimate: 3.635  (days-weeks)

 Advection Times (hr):
    Air:      100
    Water:    1000
    Sediment: 5e+004
```

Conclusion :

If released into water, regardless of the charged form, the amount distributing to air and soil will be negligible. Depending on the prevailing pH, the material will distribute into water and sediment with water being favored at lower pH levels and sediment at higher pH values.

Reliability : (2) valid with restrictions

Estimates made by an accepted method are assigned a reliability score of 2.

Flag : Critical study for SIDS endpoint
08.01.2004 (6)

3.5 BIODEGRADATION

4.1 ACUTE/PROLONGED TOXICITY TO FISH

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	= 380 mg/kg bw
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	
Vehicle	:	other: Corn oil
Doses	:	
Method	:	
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Method :

Groups of five young adult Sprague-Dawley rats (2 or 3 of each sex) were administered test material by intragastric intubation as a 25% solution in corn oil. At dosing, average group weight of males was 205 to 220 g and average group weight of females was 210-235 grams. Surviving animals were observed for 14 days and were sacrificed and necropsied.

Result :

Dose levels and results are given in the table

MALES and FEMALES

Dose	Mortality		
mg/kg	Males	Females	Combined
251	0/2	0/3	0/5
316	1/3	0/2	1/5
398	2/2	1/3	3/5
501	2/3	1/2	3/5
631	1/2	3/3	4/5
794	3/3	2/2	5/5

CLINICAL EFFECTS:
Mortality occurred from several hours to 6 days after dosing with most in four days

@ Lethal Doses: increasing weakness, collapse and death.

@ Nonlethal Doses: reduced appetite and activity for 3 to 9 days.

GROSS NECROPSY FINDINGS

Decedents: Hemorrhagic areas of lungs, liver discoloration and gastrointestinal inflammation.

Survivors: Lung congestion, viscera appeared normal at sacrifice.

Conclusion :

TBHMD has an acute oral LD50 in Sprague-Dawley rats of 380 mg/kg (95% CI 330 - 430). Males and females are approximately equally sensitive.

Reliability : (2) valid with restrictions

Study protocol was comparable to current OECD guideline, study not conducted under GLP.

Flag : Critical study for SIDS endpoint

01.11.2004 (9)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Value	:	ca. 480 mg/kg bw
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	male/female
Number of animals	:	4
Vehicle	:	other: none
Doses	:	
Method	:	
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Method :

One New Zealand albino rabbit of alternating sex per group was dermally exposed to undiluted test material at 4 dose levels. The test material remained in contact with the skin for 24 hours and was then removed. Surviving animals were observed for 14 days then were sacrificed and necropsied.

Result :

Dose levels and mortality results are given in the table

MALES and FEMALES

Dose mg/kg	Sex	Mortality	Time of death
251	F	0/1	
398	M	0/1	
631	F	1/1	one day
1000	M	1/1	one day

CLINICAL EFFECTS:
@ Lethal Doses: Rapidly increasing weakness, collapse and death.

@ Nonlethal Doses: reduced appetite and activity for 2 to 5 days.

GROSS NECROPSY FINDINGS

Decedents: Hemorrhagic areas of lung, liver discoloration, enlarged gall bladders, darkened spleen and gastrointestinal inflammation.

Survivors: Viscera appeared normal at sacrifice.

Conclusion	:	TBHMD is acutely toxic to rabbits by the dermal route with an acute dermal LD50 in rabbits between 398 and 631 mg/kg (geometric mean 480 mg/kg).
Reliability	:	(2) valid with restrictions Although the number of animals per group was small, sufficient data were generated by a scientifically defensible method to consider this a reliable estimate of dermal toxicity.
Flag	:	Critical study for SIDS endpoint

01.11.2004 (9)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	13 weeks
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	2, 5 or 20 mg/kg
Control group	:	yes, concurrent vehicle
NOAEL	:	= 2 mg/kg bw
LOAEL	:	= 5 mg/kg bw
Method	:	
Year	:	
GLP	:	yes
Test substance	:	

Method :

A 13-week corn-oil gavage study was conducted using Charles River CD rats(Charles River Breeding Laboratories, Inc., Portage, Michigan) approximately 6 weeks old at initiation. Groups of 15 animals of each sex were formed by randomly assigning animals using a computerized random selection in a block design based on body weights.

Animals were individually housed in wire-mesh cages in an environmentally controlled room. Fluorescent lighting provided illumination 12 hours per day. Water and diet were available ad libitum except during fasting for clinical pathology testing when food, but not water, was withheld. All animals were observed for overt signs of toxicity, moribundity and mortality twice daily. Detailed observations were conducted once weekly. Individual body weights and food consumption values recorded weekly. Test article was administered daily by corn-oil gavage at dose levels of 0, 2, 5 or 20 mg/kg body weight. Doses were adjusted weekly to the most recently obtained body weight.

CLINICAL PATHOLOGY: Laboratory tests were run on 10 randomly selected rats/sex/group at 13 weeks of study. The blood samples were obtained via puncture of the orbital sinus plexus from rats fasted overnight

(approximately 17 hours). Urine samples were collected during this 17-hour fasting period from rats housed individually in stainless steel metabolism cages

HEMATOLOGY PARAMETERS
Hematocrit value, hemoglobin concentration, erythrocyte count, MCH (calculated), MCV (calculated), MCHC (calculated), leukocyte count (total and differential), platelet count, reticulocyte count.

BIOCHEMISTRY PARAMETERS: Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, glucose, urea nitrogen, total bilirubin, cholesterol,
albumin, globulin (calculated), total protein, creatinine, electrolytes (sodium, potassium, chloride and calcium), phosphorus, ornithine carbamoyltransferase, gamma glutamyl transpeptidase, creatine phosphokinase.

URINALYSIS: Volume, color and appearance, pH, specific gravity, protein, glucose, ketones, urobilinogen, nitrites, bilirubin, occult blood, microscopy of spun deposit

NECROPSY: All animals were euthanized by carbon dioxide asphyxiation and received a complete post mortem examination under the direct supervision of a pathologist.

ORGAN WEIGHTS: Organs were weighed at terminal sacrifice only. Weights were recorded for liver, kidney (2), heart, adrenals (2), ovary(2),testis (2), brain.

HISTOPATHOLOGY: On all animals in the control and 20 mg/kg/day group sacrificed at study termination. Tissues fixed in formalin except eyes, which were fixed in a glutaraldehyde fixative, and stained with hematoxylin and eosin. All tissue masses and gross lesions were examined on all animals. Adrenal, liver, kidney and lung tissue of animals in the 2 and 5 mg/kg/day groups.

TISSUES PROCESSED: Adrenal (2), bone (femur), bone marrow (femur), bone marrow smear, brain (3 levels: fore, mid and hind brain), eye (2), gastrointestinal tract: esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum; gonads: ovary (2), testis with epididymis (2); heart, kidney (2), liver (2 sections), lung with mainstem bronchi (2), lymph nodes: mediastinal and mesenteric; mammary region (females only), pancreas, pituitary, prostate and seminal vesicle (2), salivary gland (mandibular with submandibular lymph node), sciatic nerve, skin, spinal cord (cervical, midthoracic and lumbar), spleen, thymic region, thyroid/ parathyroid complex, trachea, urinary bladder, uterus.

STATISTICS: Body weights (weeks 0-13), food consumption (weeks 1-13), clinical laboratory values (week 13) and organ weight (absolute and relative to body and brain weight, terminal sacrifice) data were analyzed using Bartlett's test for homogeneity of variance and analysis of variance (one-way classification). Treatment groups were compared to the control group, by sex, using the appropriate t-statistic (equal or unequal variance), as described by Steel, Torriel and Ostle. Dunnett's multiple comparison tables were used to determine significance. Total bilirubin, chloride, gamma glutamyl transpeptidase, ornithine carbamoyltransferase and specific

gravity were analyzed using a nonparametric approach, by transforming the data to ranks prior to analysis, as described by Conover and Iman. All statistical tests were two-tailed, with $p<0.05$ and $p<0.01$ used as levels of significance.

Result :

TEST SOLUTIONS: Test solutions analyzed during the study were within 10% of the target test article concentration. The mean concentration of all the analyzed solutions ranged from 98 to 105% of the desired levels. Solutions of tetrabutylhexamethylene diamine in corn oil stored for 24 hours or 9 days at room temperature were found to be stable.

SURVIVAL: All animals survived to the terminal sacrifice.

BODY WEIGHTS: Group mean body weights were statistically significantly lower than those of the vehicle control group for male rats in the 20 mg/kg-day dosage level group at week 1, and males and females in the 20 mg/kg-day dosage level group at weeks 2-13. There were also statistically significantly lower group mean body weight values for females in the 5 mg/kg-day dosage level group at weeks 12 and 13. There were no statistically significant differences in group mean body weights for males in the 2 and 5 mg/kg-day dosage level groups as compared to the male vehicle control group. With the exception of week 11, mean body weights in the 2 mg/kg-day females were not statistically different from the vehicle control mean weights.

BODY WEIGHTS AT TERMINATION (percent difference from control)

DOSE (mg/kg)	**males (g)**	**females (g)**
0	518	286
2	530 (+ 2.3)	272 (-4.9)
5	497 (- 4.0)	269* (-5.9)
20	338 *(-34.7)	205* (-28.3)

* p < 0.5

Clear test article related adverse effects were seen at the high-dose level (20 mg/kg/day). Severe weight gain depression and decreased food consumption were seen in both males and females. There were several other effects noted in the clinical pathology, organ weight and histopathology data which clearly indicated that the target organ of toxicity was the liver. At week-13, males had elevated alanine and aspartate aminotransferases and females showed elevated alanine and aspartate aminotransferases, alkaline phosphatase and cholesterol. Liver weights relative to body weights were elevated in animals of each sex (statistically significant only in females) and microscopic examinations indicated definite liver toxicity including cellular hypertrophy (6/15 males, 15/15 females) and toxic hepatitis (2/15 males, 15/15 females). Lesions characterizing the toxic hepatitis included multifocal inflammatory cell infiltration within lobules and portal triads, hepatocyte degeneration including cytoplasmic vacuolation and necrosis, increased mitosis and bile duct proliferation.

The same microscopic findings seen in the high-does group were seen in a few females in the 5 mg/kg-day group (hypertrophy 3/15 and toxic hepatitis 2/15). These findings were less severe then in the high-dose females and there were no correlative changes in serum biochemistry. In addition, females in the 5 mg/kg/day group had decreased body weights at week 13

(5.9% lower than the control mean) although this was not as severe as in the 20 mg/kg/-ay group females (28.3% lower than controls).

Both males and females in the 20 mg/kg/day groups also had elevated adrenal weights relative to body weights and microscopic evidence of trace to mild hypertrophy of cortical calls (8/15 males, 7/15 females). This was considered stress related and not a direct effect of the test article. Several other organ weight differences were noted, but there were no histopathological changes noted in any of these tissues. Therefore, these changes were likely a result of the body weight differences between the high dose and control animals.

GROSS EXAMINATION: No test article related macroscopic changes were observed among any of the terminally sacrificed male or female rats from the treatment groups.

LIVER HISTOPATHOLOGY RESULTS: Test article related microscopic changes were observed in the liver of male rats from the high dosage group and among female rats from the high and mid dosage groups. The liver changes consisted of toxic hepatitis and hepatocellular hypertrophy. The table shows the severity gradings of these changes by sex and group:

	MALES				FEMALES			
Dosage Level	0	2	5	20	0	2	5	20
Liver								
No. examined	15	15	15	15	15	15	15	15
Hepatitis, toxic,				(2)			(2)	(15)
Trace				2			2	2
mild								11
moderate								2
Hypertrophy, hepatocellular				(6)			(3)	(15)
Trace				6			3	2
Mild								11
Moderate								2

() = total number with lesion

The incidence of liver microscopic changes was minimal in the male rats in which only the high dosage group was affected. They were more pronounced in the females, in which both the high and mid dosage groups were affected.

TOXIC HEPATITIS was defined as multifocal inflammatory cell infiltration within lobules and portal triads, hepatocyte degeneration which included cytoplasmic vacuolation and necrosis (single cell or groups of cells), increased mitosis of hepatocytes and bile duct proliferation. All or some of these changes occurred in individual cases, depending upon the severity.

HEPATOCELLULAR HYPERTROPHY was defined as an increase in size of hepatic cells due to an increase in the size of the cytoplasmic compartment.

In addition to the hepatic lesions, trace to mild adrenal cortical cell hypertrophy was observed in high-dose male and female rats due to increased cytoplasmic accumulation of lipids. This was considered by the pathologist as a physiologic response resulting from non-specific stress and not directly related to the test article.

HEMATOLOGY: No test article related hematological changes were observed in the 2 and 5 mg/kg-day groups at termination. In the 20 mg/kg-day group, elevated leukocytes, characterized by elevations in both segmented neutrophils and lymphocytes were seen in animals of each sex. Other parameters were not affected, and in a few instances where statistical significance was seen, the differences were not considered to be of any biological significance.

BIOCHEMISTRY: No test article related biochemical changes occurred in the 2 or 5 mg/kg-day groups. Several biochemical parameters were affected in the 20 mg/kg-day group. Alanine aminotransferase was elevated in both males and females; the elevations in females were more pronounced. Females also showed elevations in aspartate aminotransferase, alkaline phosphatase, cholesterol and ornithine carbamoyltransferase. These findings correlate with the microscopic findings of toxic hepatitis and hepatocellular hypertrophy in these animals, and in particular, with the increased incidence and severity of liver changes seen in the females. Males additionally had decreases in creatinine, total protein, albumin and glucose when compared to control values. Decreases in total protein glucose and albumin could have occurred from both liver disease and malnutrition. Decreases in creatinine are occasionally seen but an exact mechanism is unknown. Decreases seen in females included creatinine and albumin.

URINALYSIS: There were no test article related changes in urinalysis values in males and females in the 2 and 5 mg/kg-day groups and males in the 20 mg/kg/day groups at the 13-week interval. Females in the 20 mg/kg-day group had an increase in urinary volume and a decrease in specific gravity. The significance of these findings is unknown.

Test substance :

Tetrabutylhexamethylenediamine (TBHMD) CASNO 27090-63-7

Conclusion :

Oral administration of test substance for 13 weeks was associated with pathological changes in the liver of 20 mg/kg-day rats of each sex. Females appeared to be more affected. At 5 mg/kg-day, females showed slight liver pathology but males were not affected. Decrease in body weight gain, increase in leukocyte count, and increases in serum enzymes indicative of an hepatotoxic effect were also seen at 20 mg/kg-day. Although effects at 5 mg/kg-day were minor, it is considered a LOAEL and 2 mg/kg-day is considered the NOAEL

Reliability : (1) valid without restriction

Guideline-like study conducted under GLPs with full documentation.

Flag : Critical study for SIDS endpoint

10.03.2005 (3)

5.5 GENETIC TOXICITY ‘IN VITRO‘

5.6 GENETIC TOXICITY ‘IN VIVO‘

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

(1) Calculated using EPIWIN 3.05 by Toxicology and Regulatory Affairs, October 2004

(2) Estimated by Toxicology and Regulatory Affairs based on accepted chemical principles. October 2004

(3) International Research and Development Corp., Final Report: Tetrahexamethylenediamine, 13-Week Oral Toxicity Study in Rats. Monsanto Study IR 83-153, Sponsored by Monsanto. April 18, 1985.

(4) EPIWIN 3.05, Syracuse Research Corporation 2000

(5) Calculation of solubility based on pKa by Toxicology and Regulatory Affairs, December 2003.

(6) Estimation made using EQC Model contained in EPIWIN 3.05 with additional inputs to accommodate the doubly charged form, by Toxicology and Regulatory Affairs, December 2003.

(7) Estimation made using KOWWIN Program (v1.66) with manual calculations to accommodate the doubly charged form, by Toxicology and Regulatory Affairs, December 2003.

(8) Solutia Material Safety Data Sheet #027090637 version of Aug 31, 1998.

(9) Younger Laboratories Inc, Final Report: Acute Toxicity Testing of N,N,N',N' Tetrabutylhexamethylene diamine project YO-75-165, 07-29-1975; sponsored by Monsanto Co.